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## **APPENDIX**

### **Part I. Claims**

A marked-up version of Claims 1-57 follows. Please amend Claims 32, 34, 35 and 50, cancel non-elected Claims 1-19, 36-49 and 51-57, without prejudice. Words that are added are underlined. Words that are deleted are enclosed in brackets. Italicized brackets are *not* deletions. Claims 20-31 and 33 are not amended and are presented as originally filed. Claim status is stated in parenthesis at the beginning of each claim.

1. (NONELECTED/CANCELED) A buffer composition for use in a high temperature hybridization assay of a microarray of oligonucleotides bound to an adsorbed polymer surface of a siliceous substrate comprising:
  - a pH within a range of pH 6.4 and 7.5;
  - a monovalent cation having a monovalent cation concentration ranging from 0.01 M and 2.0 M; and
  - a non-chelating buffering agent that maintains the pH within the range.
2. (NONELECTED/CANCELED) The buffer composition of Claim 1, wherein the non-chelating buffering agent is selected from a group consisting of 2-[*N*-morpholino]ethanesulfonic acid (MES), 3-(*N*-Morpholine)propanesulfonic acid (MOPS), Piperazine-*N,N'*-bis(2-ethanesulfonic acid (PIPES), Tris(hydroxymethyl)aminomethane hydrochloride (TRIS-HCl), Hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), and *N*-Tris(hydroxymethyl)methylglycine (TRICINE).
3. (NONELECTED/CANCELED) The buffer composition of Claim 1, wherein the monovalent cation is selected from a group consisting of LiCl, NaCl and KCl, and the monovalent cation concentration ranges from about 0.10 M to about 2.0 M.

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4. (NONELECTED/CANCELED) The buffer composition of Claim 1, further comprising a total cation concentration that ranges from about 0.02 M to about 2.0 M.

5. (NONELECTED/CANCELED) The buffer composition of Claim 1, further comprising a chelating agent selected from a group consisting of one or more of ethylenediaminetetraacetic acid (EDTA), trans-1,2-diaminocyclohexanetetraacetic acid (CDTA) and diethylenetriaminopentaacetic acid (DTPA), having a chelating agent concentration of less than about 100  $\mu$ M.

6. (NONELECTED/CANCELED) The buffer composition of Claim 1, further comprising an ionic surfactant selected from a group consisting of one or more of sodium dodecyl sulfate (SDS), lithium lauryl sulfate (LLS), N-lauryl sarcoside, acylated polypeptides, linear alkybenzene sulfonates, lignin sulfonates, paraffin sulfonates, sulfosuccinate esters, alkylnaphthalene sulfonates, isethionates, alkanolamine condensates, N-alkylpyrrolidones, and sarcosinate-derived surfactants, wherein the ionic surfactant has a surfactant concentration ranging from about 0.01 % to 0.2 % (w/v).

7. (NONELECTED/CANCELED) The buffer composition of Claim 1, further comprising an ionic surfactant selected from a group consisting of one or more of sodium dodecyl sulfate (SDS) and lithium lauryl sulfate (LLS) and the amount of the ionic surfactant is a surfactant concentration that is less than or equal to about 0.1 % (w/v).

8. (NONELECTED/CANCELED) The buffer composition of Claim 1, wherein the adsorbed polymer surface comprises a polycationic polymer.

9. (NONELECTED/CANCELED) The buffer composition of Claim 8, wherein the polycationic polymer is selected from a group consisting of one or more of polyethylenediamine, poly-acrylamide, poly-L-arginine, poly-L-histidine, and poly-L-lysine.

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10. (NONELECTED/CANCELED) The buffer composition of Claim 1, wherein the non-chelating buffering agent is 2-[*N*-morpholino]ethanesulfonic acid (MES), the monovalent cation is LiCl, the monovalent cation concentration ranges from 0.1 M to 2.0 M, and the pH is within the range of 6.6 to 6.8.

11. (NONELECTED/CANCELED) The buffer composition of Claim 10, optionally further comprising one or both of a chelating agent and an ionic surfactant, wherein the chelating agent is ethylenediaminetetraacetic acid (EDTA) having a chelating agent concentration of about 50  $\mu$ M, and the ionic surfactant is selected from a group consisting of one or more of sodium dodecyl sulfate (SDS) and lithium lauryl sulfate (LLS), having a surfactant concentration of less than or equal to about 0.1 % (w/v), and wherein the buffer composition has a total cation concentration of about 750 mM.

12. (NONELECTED/CANCELED) A buffer composition for use in hybridizing a nucleic acid material in a high temperature hybridization assay using a siliceous array substrate having an adsorbed polymer surface for attaching nucleic acids comprising:

- a pH within a range of pH 6.4 and 7.5;
- a non-chelating buffering agent that maintains the pH within the pH range;
- a monovalent cation;
- optionally one or both of a chelating agent that chelates divalent cations and an ionic surfactant; and
- a total cation concentration that ranges from about 0.02 M to about 2.0 M.

13. (NONELECTED/CANCELED) The buffer composition of Claim 12, wherein the non-chelating buffering agent is selected from a group consisting of 2-[*N*-morpholino]ethanesulfonic acid (MES), 3-(*N*-Morpholine)propanesulfonic acid (MOPS), Piperazine-*N,N'*-bis(2-ethanesulfonic acid (PIPES), Tris(hydroxymethyl)aminomethane hydrochloride (TRIS-HCl),

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Hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid (HEPES), and *N*-Tris(hydroxymethyl)methylglycine (TRICINE).

14. (NONELECTED/CANCELED) The buffer composition of Claim 12, wherein the monovalent cation is selected from a group consisting of LiCl, NaCl and KCl, and has a monovalent cation concentration that ranges from about 0.01 M to about 2.0 M.

15. (NONELECTED/CANCELED) The buffer composition of Claim 12, wherein the optional chelating agent is selected from a group consisting of one or more of ethylenediaminetetraacetic acid (EDTA), trans-1,2-diaminocyclohexanetetraacetic acid (CDTA) and diethylenetriaminopentaacetic acid (DTPA) and has a chelating agent concentration of less than about 100  $\mu$ M; and

wherein the optional ionic surfactant is selected from a group consisting of one or more of sodium dodecyl sulfate (SDS), lithium lauryl sulfate (LLS), N-lauryl sarcoside, acylated polypeptides, linear alkybenzene sulfonates, lignin sulfonates, paraffin sulfonates, sulfosuccinate esters, alkylnaphthalene sulfonates, isethionates, alkanolamine condensates, N-alkylpyrrolidones, and sarcosinate-derived surfactants, and has a surfactant concentration ranging from about 0.01 % to about 0.2% (w/v).

16. (NONELECTED/CANCELED) The buffer composition of Claim 12, wherein the adsorbed polymer surface comprises a polycationic polymer.

17. (NONELECTED/CANCELED) The buffer composition of Claim 16, wherein the polycationic polymer is selected from a group consisting of one or more of polyethylenediamine, poly-acrylamide, poly-L-arginine, poly-L-histidine, and poly-L-lysine.

18. (NONELECTED/CANCELED) The buffer composition of Claim 12, wherein the non-chelating buffering agent is 2-[*N*-morpholino]ethanesulfonic acid (MES), the monovalent cation is LiCl in a monovalent cation concentration that ranges from about 0.1 M to about 2.0 M, and the pH is within the range of pH 6.6 to

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6.8, and wherein the optional chelating agent is ethylenediaminetetraacetic acid (EDTA) having a chelating agent concentration of about 50  $\mu$ M, and the optional ionic surfactant is selected from a group consisting of one or more of sodium dodecyl sulfate (SDS) and lithium lauryl sulfate (LLS), having a surfactant concentration of less than or equal to about 0.1 % (w/v), and wherein the total cation concentration ranges from about 0.1 M to about 2.0 M.

19. (NONELECTED/CANCELED) The buffer composition of Claim 12, further comprising nucleic acids in solution that are hybridized with the nucleic acid material on the array substrate.

20. (NOT AMENDED) A method of hybridizing a microarray of oligonucleotides bound to an adsorbed polymer surface on a siliceous substrate with a nucleic acid material comprising the step of:

incubating the nucleic acid material with the microarray of oligonucleotides on the adsorbed polymer surface in a hybridization solution at a hybridization temperature ranging from about 55°C to about 70°C so as to hybridize the nucleic acid material,

wherein the hybridization solution comprises a buffer composition that comprises a pH within a range of pH 6.4 to 7.5, a non-chelating buffering agent that maintains the pH within the pH range, and a monovalent cation in a monovalent cation concentration ranging from about 0.01 M to about 2.0 M.

21. (NOT AMENDED) The method of Claim 20, wherein in the step of incubating, the non chelating buffering agent is selected from a group consisting of 2-[*N*-morpholino]ethanesulfonic acid (MES), 3-(*N*-Morpholine)propanesulfonic acid (MOPS), Piperazine-*N,N'*-bis(2-ethanesulfonic acid (PIPES), Tris(hydroxymethyl)aminomethane hydrochloride (TRIS-HCl), Hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), and *N*-Tris(hydroxymethyl)methylglycine (TRICINE).

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22. (NOT AMENDED) The method of Claim 20, wherein in the step of incubating, the monovalent cation is selected from a salt consisting of one or more of LiCl, NaCl and KCl and the monovalent cation concentration ranges from about 0.1 M to about 2.0 M.

23. (NOT AMENDED) The method of Claim 20, wherein the adsorbed polymer surface comprises a polycationic polymer.

24. (NOT AMENDED) The method of Claim 23, wherein the polycationic polymer is selected from a group consisting of one or more of polyethylenediamine, poly-acrylamide, poly-L-arginine, poly-L-histidine, and poly-L-lysine.

25. (NOT AMENDED) The method of Claim 20, wherein in the step of incubating, the buffer composition further comprises a chelating agent selected from a group consisting of one or more of ethylenediaminetetraacetic acid (EDTA), trans-1, 2-diaminocyclohexanetetraacetic acid (CDTA) and diethylenetriaminopentaacetic acid (DTPA) that has a chelating agent concentration of less than about 100  $\mu$ M.

26. (NOT AMENDED) The method of Claim 20, wherein in the step of incubating, the buffer composition further comprises an ionic surfactant selected from a group consisting of one or more of sodium dodecyl sulfate (SDS), lithium lauryl sulfate (LLS), N-lauryl sarcoside, acylated polypeptides, linear alkybenzene sulfonates, lignin sulfonates, paraffin sulfonates, sulfosuccinate esters, alkylnaphthalene sulfonates, isethionates, alkanolamine condensates, and N-alkylpyrrolidones, and wherein the step of incubating comprises using a hybridization chamber, and the ionic surfactant is provided in an amount sufficient to wet surfaces of the hybridization chamber and loosen bubbles impinged on the surfaces of the hybridization chamber.

27. (NOT AMENDED) The method of Claim 26, wherein the amount of ionic surfactant is a surfactant concentration ranging from about 0.01% to about 0.2% (w/v).

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28. (NOT AMENDED) The method of Claim 20, wherein the buffer composition has a total cation concentration of about 0.02 M to about 2.0 M.

29. (NOT AMENDED) The method of Claim 20, wherein in the step of incubating, the non chelating buffering agent is 2-[N-morpholino]ethanesulfonic acid (MES), the monovalent cation is LiCl, the monovalent cation concentration is greater than or equal to 300 mM, the pH is within the range of pH 6.6 to 6.8.

30. (NOT AMENDED) The method of Claim 29, wherein in the step of incubating, the buffer composition further comprises one or both of a chelating agent ethylenediaminetetraacetic acid EDTA having a chelating agent concentration of about 50  $\mu$ M, and an ionic surfactant selected from sodium dodecyl sulfate (SDS), lithium lauryl sulfate (LLS) having a surfactant concentration that ranges from about 0.02 % to about 0.1 % (w/v), and the buffer composition has a total cation concentration of about 750 mM.

31. (NOT AMENDED) The method of Claim 20, before the step of incubating, further comprising the step of combining the nucleic acid material with the buffer composition.

32. (AMENDED) The method of Claim 20, after the step of incubating, further comprising the step of interrogating the hybridized microarray at a first location, the first location being a physical location either where the incubation of the microarray is performed or another location separate from the microarray incubation location.

33. (NOT AMENDED) The method of Claim 32, further comprising the step of transmitting data representing a result of the interrogation.

34. (AMENDED) The method of Claim 33, further comprising the step of receiving the transmitted data at a second location, the second location being a physical location that is different from one or both of the first location where the microarray interrogation is performed and the microarray incubation location.



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35. (AMENDED) The method of Claim 34, wherein the first location is remote from the second location, the remote first location being physically separated from the second location.

36. (NONELECTED/CANCELED) A buffer kit for a hybridization assay of a nucleic acid material that uses a siliceous substrate having a surface adsorbed polymer on a surface of the substrate for attaching nucleic acids thereto comprising:

a buffer composition for use in the hybridization assay that comprises a pH within a range of pH 6.4 and 7.5; a non-chelating buffering agent that maintains the pH within the pH range; a monovalent cation; optionally one or both of a chelating agent that chelates divalent cations and an ionic surfactant; and a total cation concentration ranging from about 0.02 M to about 2.0 M.

37. (NONELECTED/CANCELED) The buffer kit of Claim 36, further comprising a nucleic acid sample for use as a hybridization assay control.

38. (NONELECTED/CANCELED) The buffer kit of Claim 36, further comprising a list of chemical components for the buffer composition.

39. (NONELECTED/CANCELED) The buffer kit of Claim 36, further comprising instructions for using the buffer composition in the hybridization assay with a microarray that comprises the siliceous substrate with the surface adsorbed polymer, and a plurality of nucleic acids attached to the surface adsorbed polymer in an array pattern of features.

40. (NONELECTED/CANCELED) The buffer kit of Claim 36, wherein the buffer composition further comprises nucleic acids in solution to hybridize with the nucleic acid material, the nucleic acid material being attached to the adsorbed polymer surface of the siliceous substrate.

41. (NONELECTED/CANCELED) The buffer kit of Claim 36, further comprising a microarray comprising the siliceous substrate with the surface adsorbed polymer, and a plurality of nucleic acids attached to the surface adsorbed polymer in



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an array pattern of features to hybridize with the nucleic acid material, and optionally further comprising instructions for using the buffer composition in the hybridization assay with the microarray at a hybridization temperature that ranges from about 55°C to about 70°C.

42. (NONELECTED/CANCELED) The buffer kit of Claim 36, wherein the non-chelating buffering agent is selected from a group consisting of 2-[*N*-morpholino]ethanesulfonic acid (MES), 3-(*N*-Morpholine)propanesulfonic acid (MOPS), Piperazine-*N,N'*-bis(2-ethanesulfonic acid (PIPES), Tris(hydroxymethyl)aminomethane hydrochloride (TRIS-HCl), Hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), and *N*-Tris(hydroxymethyl)methylglycine (TRICINE).

43. (NONELECTED/CANCELED) The buffer kit of Claim 36, wherein the monovalent cation is selected from a group consisting of one or more of LiCl, KCl, and NaCl and has a monovalent cation concentration ranging from about 0.01 M to about 2.0 M.

44. (NONELECTED/CANCELED) The buffer kit of Claim 36, wherein the optional chelating agent is selected from a group consisting of one or more of ethylenediaminetetraacetic acid (EDTA), trans-1, 2-diaminocyclohexanetetraacetic acid (CDTA) and diethylenetriaminopentaacetic acid (DTPA), having a chelating agent concentration of less than about 100 µM; and

wherein the optional ionic surfactant is selected from a group consisting of one or more of sodium dodecyl sulfate (SDS), lithium lauryl sulfate (LLS), *N*-lauryl sarcoside, acylated polypeptides, linear alkybenzene sulfonates, lignin sulfonates, paraffin sulfonates, sulfosuccinate esters, alkylnaphthalene sulfonates, isethionates, alkanolamine condensates, and *N*-alkylpyrrolidones, other sulfonate-containing surfactants, such as sarcosinate-derived surfactants, and other amine-containing surfactants, having a surfactant concentration ranging from about 0.01 % to about 0.20 % (w/v).

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45. (NONELECTED/CANCELED) The buffer kit of Claim 36, wherein the surface adsorbed polymer comprises a polycationic polymer.

46. (NONELECTED/CANCELED) The buffer kit of Claim 45, wherein the polycationic polymer is selected from a group consisting of one or more of polyethylenediamine, poly-acrylamide, poly-L-arginine, poly-L-histidine, and poly-L-lysine.

47. (NONELECTED/CANCELED) A kit comprising the buffer composition of Claim 1, and optionally further comprising:

one or more of the microarray; a sample of nucleic acids for use as a hybridization assay control; and instructions for using the buffer composition in the hybridization assay.

48. (NONELECTED/CANCELED) A kit comprising the buffer composition of Claim 12 and optionally further comprising:

one or more of a microarray of oligonucleotides attached to the adsorbed polymer surface of the siliceous array substrate; a sample of nucleic acids for use as a hybridization assay control; and instructions for using the buffer composition in the hybridization assay with one or both of the optional microarray or the nucleic acid material attached to the adsorbed polymer surface of the siliceous array substrate.

49. (NONELECTED/CANCELED) A buffer kit for use in a hybridization assay according to the method of Claim 20 comprising:

the buffer composition that optionally further comprises one or both of a chelating agent that chelates divalent cations and an ionic surfactant, and has a total cation concentration ranging from about 0.02 M to about 2.0 M; and

optionally comprising a nucleic acid sample as a hybridization assay control.

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50. (AMENDED) A method of performing a high temperature hybridization assay [with the buffer composition of Claim 1] comprising the step of:

incubating [the] a nucleic acid material with [the] a microarray of oligonucleotides [on the adsorbed polymer surface] in [the buffer composition] a hybridization solution at a hybridization temperature ranging from about 55°C to about 70°C so as to hybridize the nucleic acid material,

wherein the microarray comprises a siliceous substrate with an adsorbed polymer surface and oligonucleotides bound to the adsorbed polymer surface, and

wherein the hybridization solution comprises a pH within a range of pH 6.4 and 7.5 and a buffer composition, the buffer composition comprising a non-chelating buffering agent that maintains the pH within the range and a monovalent cation having a monovalent cation concentration ranging from 0.01 M and 2.0 M.

51. (NONELECTED/CANCELED) A kit for a hybridization assay of a nucleic acid material with an array that comprises an adsorbed polymer surface on a siliceous substrate, the kit comprising:

a list of chemical components for a buffer composition that comprises a pH within a range of pH 6.4 and 7.5, a non-chelating buffering agent that maintains the pH within the pH range, a monovalent cation, and a total cation concentration ranging from about 0.02 M to about 2.0 M for use in the hybridization assay.

52. (NONELECTED/CANCELED) The kit of Claim 51, wherein the list of components for the buffer composition comprises:

the non-chelating buffering agent selected from a group consisting of 2-[N-morpholino]ethanesulfonic acid (MES), 3-(N-Morpholine)propanesulfonic acid (MOPS), Piperazine-N,N'-bis(2-ethansulfonic acid (PIPES), Tris(hydroxymethyl)aminomethane hydrochloride (TRIS-HCl), Hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), and N-Tris(hydroxymethyl)methylglycine (TRICINE); and

the monovalent cation selected from a group consisting of one or more of LiCl, KCl, and NaCl; and

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optionally further comprises:

a chelating agent selected from a group consisting of one or more of ethylenediaminetetraacetic acid (EDTA), trans-1, 2-diaminocyclohexanetetraacetic acid (CDTA) and diethylenetriaminopentaacetic acid (DTPA); and

an ionic surfactant selected from a group consisting of one or more of sodium dodecyl sulfate (SDS), lithium lauryl sulfate (LLS), N-lauryl sarcoside, acylated polypeptides, linear alkybenzene sulfonates, lignin sulfonates, paraffin sulfonates, sulfosuccinate esters, alkylnaphthalene sulfonates, isethionates, alkanolamine condensates, and N-alkylpyrrolidones, other sulfonate-containing surfactants, such as sarcosinate-derived surfactants, and other amine-containing surfactants.

53. (NONELECTED/CANCELED) The kit of Claim 51, wherein the monovalent cation has a monovalent cation concentration that ranges from about 0.01 M to about 2.0 M, and the buffer composition optionally further comprises one or both of a chelating agent that has a chelating agent concentration of less than about 100  $\mu$ M; and an ionic surfactant that has a surfactant concentration ranging from about 0.01 % to about 0.20 % (w/v).

54. (NONELECTED/CANCELED) The kit of Claim 51, wherein the pH of the buffer composition is within the range of pH 6.6 to 6.8, the non-chelating buffering agent is 2-[N-morpholino] ethanesulfonic acid (MES), the monovalent cation is LiCl having the monovalent cation concentration ranging from about 0.1 M to about 2.0 M, and wherein the buffer composition optionally further comprises one or both of ethylenediaminetetraacetic acid (EDTA) chelating agent having the chelating concentration equal to about 50  $\mu$ M, and an ionic surfactant is selected from the group consisting of sodium dodecyl sulfate (SDS) and lithium lauryl sulfate (LLS) having a surfactant concentration ranging from about 0.02 % to about 0.10 % (w/v), and wherein the total cation concentration of the buffer composition ranges from about 0.1 M to about 2.0 M.

55. (NONELECTED/CANCELED) The kit of Claim 51, further comprising the buffer composition, and optionally further comprising instructions for using the

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buffer composition with the array in the hybridization assay to hybridize the nucleic acid material at a temperature ranging from about 55°C to about 70°C, the array having the siliceous substrate with the surface adsorbed polymer, and a plurality of nucleic acids attached to the surface adsorbed polymer in an array pattern of features to hybridize with the nucleic acid material.

56. (NONELECTED/CANCELED) The kit of Claim 51, wherein the surface adsorbed polymer comprises a polycationic polymer.

57. (NONELECTED/CANCELED) The kit of Claim 56, wherein the polycationic polymer is selected from a group consisting of one or more of polyethylenediamine, poly-acrylamide, poly-L-arginine, poly-L-histidine, and poly-L-lysine.

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